



#7

SEQUENCE LISTING

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Gregory, Philip

<120> MODULATION OF ENDOGENOUS GENE EXPRESSION IN CELLS

<130> 8325-0002.21 / S2-US5

<140> 09/942,087

<141> 2001-08-28

<150> 09/229,037

<151> 1999-01-12

<160> 43

<170> PatentIn Ver. 2.0

<210> 1

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary motif
of C2H2 class of zinc finger proteins (ZFP)

<220>

<221> MOD_RES

<222> (2)..(3)

<223> Xaa = any amino acid

<220>

<221> MOD_RES

<222> (4)..(5)

<223> Xaa = any amino acid, may be present or absent

<220>

<221> MOD_RES

<222> (7)..(18)

<223> Xaa = any amino acid

<220>

<221> MOD_RES

<222> (20)..(22)

<223> Xaa = any amino acid

<220>

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<222> (23)..(24)

<223> Xaa = any amino acid, may be present or absent

<400> 1

Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

1	5	10	15
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Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His
 20 25

<210> 2
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ZFP target site
with two overlapping D-able subsites

<220>
<221> modified_base
<222> (1)..(2)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (5)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (8)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (9)
<223> n = a,c or t; if g, then position 10 cannot be g
or t

<220>
<221> modified_base
<222> (10)
<223> n = a or c; if g or t, then position 9 cannot be g

<400> 2
nngkngknnn

10

<210> 3
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ZFP target site
with three overlapping D-able subsites

<220>
<221> modified_base
<222> (1)..(2)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (5)

<223> n = g,a,c or t

<220>

<221> modified_base

<222> (8)

<223> n = g,a,c or t

<400> 3

nngkngkngk

10

<210> 4

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 4

Asp Gly Gly Gly Ser
1 5

<210> 5

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 5

Thr Gly Glu Lys Pro
1 5

<210> 6

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 6

Leu Arg Gln Lys Asp Gly Glu Arg Pro
1 5

<210> 7

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 7

Gly Gly Arg Arg
1

<210> 8
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 8
Gly Gly Gly Gly Ser
1 5

<210> 9
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 9
Gly Gly Arg Arg Gly Gly Gly Ser
1 5

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 10
Leu Arg Gln Arg Asp Gly Glu Arg Pro
1 5

<210> 11
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 11
Leu Arg Gln Lys Asp Gly Gly Gly Ser Glu Arg Pro
1 5 10

<210> 12
<211> 16
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 12

Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro
1 5 10 15

<210> 13

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP target site
region surrounding initiation site of vascular
endothelial growth factor (VEGF) gene containing
two 9-base pair target sites

<220>

<221> protein_bind

<222> (4)..(12)

<223> upstream 9-base pair ZFP VEGF1 target site

<220>

<221> protein_bind

<222> (14)..(22)

<223> downstream 9-base pair ZFP VEGF3a target site

<400> 13

agcggggagg atcgcgagg cttgg

25

<210> 14

<211> 298

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:VEGF1 ZFP
construct targeting upstream 9-base pair target
site in VEGF promoter

<220>

<221> CDS

<222> (2)..(298)

<223> VEGF1

<400> 14

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97
Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly

35	40	45	
aaa cgc ttc acc cgt tgc tca aac ctg cag cgt cac aag cgt acc cac			193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His			
50	55	60	
acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg			241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met			
65	70	75	80
cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag			289
Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys			
	85	90	95
ggt gga tcc			298
Gly Gly Ser			

<210> 15
 <211> 99
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF1 ZFP
 construct targeting upstream 9-base pair target
 site in VEGF promoter

<400> 15
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15
Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
20 25 30
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
35 40 45
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
50 55 60
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
65 70 75 80
Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95
Gly Gly Ser

<210> 16
 <211> 298
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF3a ZFP
 construct targeting downstream 9-base pair target
 site in VEGF promoter

<220>
 <221> CDS
 <222> (2)..(298)
 <223> VEGF3a

<400> 16

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g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
  Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
    1             5             10             15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
          20             25             30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
          35             40             45

aaa cgc ttc acc cgt tcg tca aac cta cag agg cac aag cgt aca cac 193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
          50             55             60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
        65             70             75             80

cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
          85             90             95

ggg gga tcc
Gly Gly Ser
                                     298

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<210> 17
 <211> 99
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:VEGF3a ZFP
 construct targeting downstream 9-base pair target
 site in VEGF promoter

<400> 17

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Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
  1             5             10             15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
          20             25             30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
          35             40             45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
          50             55             60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
        65             70             75             80

```

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95

Gly Gly Ser

<210> 18
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA target
site 1 recognition (top) strand

<220>
<221> protein_bind
<222> (11)..(19)
<223> VEGF DNA ZFP target site 1

<400> 18
catgcatagc ggggagggatc gccatcgat 29

<210> 19
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA site 1
complementary (bottom) strand

<400> 19
atcgatggcg atcctccccg ctatgcatg 29

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA
target site 3 recognition (top) strand

<220>
<221> protein_bind
<222> (11)..(19)
<223> VEGF DNA ZFP target site 3

<400> 20
catgcatatc gcggaggctt ggcacgat 29

<210> 21
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF DNA target
 site 3 complementary (bottom) strand

<400> 21
 atcgatgcca agcctccgcg atatgcatg 29

<210> 22
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer SPE7

<400> 22
 gagcagaatt cggcaagaag aagcagcac 29

<210> 23
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer SPEamp12

<400> 23
 gtggtctaga cagctcgtca cttcgc 26

<210> 24
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer SPE
 amp13

<400> 24
 ggagccaagg ctgtggtaaa gtttacgg 28

<210> 25
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer SPEamp11

<400> 25
 ggagaagctt ggatcctcat tatccc 26

<210> 26
 <211> 83
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:sequence
ligated between XbaI and StyI sites

<400> 26

tctagacaca tcaaaaccca ccagaacaag aaagacggcg gtggcagcgg caaaaagaaa 60

cagcacatat gtcacatcca agg

83

<210> 27

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer GB19

<400> 27

gccatgccgg tacccatacc tggcaagaag aagcagcac

39

<210> 28

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer GB10

<400> 28

cagatcggat ccacccttct tattctggtg ggt

33

<210> 29

<211> 589

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:designed
6-finger ZFP VEGF3a/1 from KpnI to BamHI

<220>

<221> CDS

<222> (2)..(589)

<223> VEGF3a/1

<400> 29

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49

Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly

1

5

10

15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg

20

25

30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly

35	40	45	
aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac			193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His			
50	55	60	
aca ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg			241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met			
65	70	75	80
cga agt gac gag ctg tct aga cac atc aaa acc cac cag aac aag aaa			289
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys			
85	90	95	
gac ggc ggt ggc agc ggc aaa aag aaa cag cac ata tgt cac atc caa			337
Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln			
100	105	110	
ggc tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg			385
Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu			
115	120	125	
cgc tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt			433
Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys			
130	135	140	
ggt aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc			481
Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr			
145	150	155	160
cac acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc			529
His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe			
165	170	175	
atg cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag			577
Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys			
180	185	190	
aag ggt gga tcc			589
Lys Gly Gly Ser			
195			

<210> 30

<211> 196

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:designed
6-finger ZFP VEGF3a/1 from KpnI to BamHI

<400> 30

Val	Pro	Ile	Pro	Gly	Lys	Lys	Lys	Gln	His	Ile	Cys	His	Ile	Gln	Gly
1				5				10						15	

Cys	Gly	Lys	Val	Tyr	Gly	Gln	Ser	Ser	Asp	Leu	Gln	Arg	His	Leu	Arg
			20				25					30			

Trp	His	Thr	Gly	Glu	Arg	Pro	Phe	Met	Cys	Thr	Trp	Ser	Tyr	Cys	Gly
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35					40					45					
Lys	Arg	Phe	Thr	Arg	Ser	Ser	Asn	Leu	Gln	Arg	His	Lys	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Lys	Phe	Ala	Cys	Pro	Glu	Cys	Pro	Lys	Arg	Phe	Met
65					70					75					80
Arg	Ser	Asp	Glu	Leu	Ser	Arg	His	Ile	Lys	Thr	His	Gln	Asn	Lys	Lys
			85						90					95	
Asp	Gly	Gly	Gly	Ser	Gly	Lys	Lys	Lys	Gln	His	Ile	Cys	His	Ile	Gln
			100					105					110		
Gly	Cys	Gly	Lys	Val	Tyr	Gly	Thr	Thr	Ser	Asn	Leu	Arg	Arg	His	Leu
	115						120					125			
Arg	Trp	His	Thr	Gly	Glu	Arg	Pro	Phe	Met	Cys	Thr	Trp	Ser	Tyr	Cys
	130					135					140				
Gly	Lys	Arg	Phe	Thr	Arg	Ser	Ser	Asn	Leu	Gln	Arg	His	Lys	Arg	Thr
145						150					155				160
His	Thr	Gly	Glu	Lys	Lys	Phe	Ala	Cys	Pro	Glu	Cys	Pro	Lys	Arg	Phe
			165					170					175		
Met	Arg	Ser	Asp	His	Leu	Ser	Arg	His	Ile	Lys	Thr	His	Gln	Asn	Lys
			180					185					190		
Lys	Gly	Gly	Ser												
	195														

<210> 31
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:JVF9 VEGF3a/1
 target oligonucleotide

<400> 31
 agcgagcggg gaggatcgcg gaggcttggg gcagccgggt ag

42

<210> 32
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:JVF10 VEGF3a/1
 target oligonucleotide complementary sequence

<400> 32
 cgctctaccc ggctgccccca agcctccgcg atcctccccg ct

42

<210> 33
 <211> 25

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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer JVF24

<400> 33
cgcggatccg cccccccgac cgatg                25
<210> 34
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:downstream
      primer JVF25

<400> 34
ccgcaagctt acttgatcatc gtcgtccttg tagtcgctgc cccaccgta ctggtcaatt 60
cc                                                                 62

<210> 35
<211> 7
<212> PRT
<213> Simian virus 40

<220>
<221> PEPTIDE
<222> (1)..(7)
<223> SV40 large T antigen nuclear localization sequence
      (NLS)

<400> 35
Pro Lys Lys Lys Arg Lys Val
  1                      5

<210> 36
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:segment from
      EcoRI to KpnI containing Kozak sequence including
      initiation codon and SV40 NLS

<400> 36
gaattcgcta ggcaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60
c                                                                 61

<210> 37
<211> 187
<212> DNA
<213> Artificial Sequence

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<220>

<223> Description of Artificial Sequence:segment from
KpnI to XhoI containing BamHI site, KRAB-A box
from KOX1, FLAG epitope and HindIII site

<400> 37

ggatcccgagg gatcccgagg actggtgacc ttcaaggatg tattgtgga cttcaccagg 60
gaggagtgga agctgctgga cactgctcag cagatcgtgt acagaaatgt gatgctggag 120
aactataaga acctggtttc cttgggcagc gactacaagg acgacgatga caagtaagct 180
tctcgag 187

<210> 38

<211> 277

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:inserted
fragment from BamHI to HindIII sites

<400> 38

ggatccgccc ccccgaccga tgtagcctg ggggacgagc tccacttaga cggcgaggac 60
gtggcgatgg cgcatgccga cgcgctagac gatttcgatc tggacatgtt gggggacggg 120
gattccccgg ggccgggatt tccccccac gactccgccc cctacggcgc tctggatatg 180
gccgacttcg agtttgagca gatgtttacc gatgcccttg gaattgacga gtacgggtggg 240
ggcagcgact acaaggacga cgatgacaag taagctt 277

<210> 39

<211> 118

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:sequence
replacing NLS-KRAB-FLAG with NLS-FLAG only

<400> 39

gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60
cccggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118

<210> 40

<211> 204

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:insert into
MluI/BglII sites of pGL3-Control to create
pVFR1-4x

<400> 40
acgcgtaagc ttgctagcga gcggggagga tcgcggaggc ttggggcagc cgggtagagc 60
gagcgggggag gatcgcggag gcttggggca gccgggtaga gcgagcgggg aggatcgcgg 120
aggcttgggg cagccgggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180
tagagcgctc agaagcttag atct 204

<210> 41
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: "D-able" site
motif

<400> 41
nngk 4

<210> 42
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: D-able site
subtype

<400> 42
nngg 4

<210> 43
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: D-able site
subtype

<400> 43
nngt 4